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Sutaryo Sutaryo[a], Alastair James Ward[b] & Henrik Bjarne Møller[b]

[a] Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang 50275, Indonesia

[b] Department of Engineering, Aarhus University, Tjele, Denmark

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Sutaryo Sutaryo[a*], Alastair James Ward[b] and Henrik Bjarne Møller[b]

[a]*Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang 50275, Indonesia;* [b]*Department of Engineering, Aarhus University, Tjele, Denmark*

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This study investigates the effect of applying a mixture of enzymes (ME) to dairy cattle manure (DCM) as substrate in anaerobic digestion (AD). The aims of this study were to evaluate different methods of ME application to DCM at different temperatures and to investigate the effect of adding ME during the pre-treatment of the solid fractions of dairy cattle manure (SFDCM). The results showed that there was no positive effect of direct ME addition to substrate at either mesophilic (35 °C) or thermophilic (50 °C) process temperatures, but there was a significant 4.44% increase in methane yield when DCM, which had been incubated with ME addition at 50 °C for three days, was fed to a digester when compared to a control digester operating at the same retention time. Methane production was detected during the pre-treatment incubation, and the total sum methane yield during pre-treatment and digestion was found to be 8.33% higher than in the control. The addition of ME to the SFDCM in a pre-incubation stage of 20 h at 35 °C gave a significant increase in methane yield by 4.15% in a digester treating a mixed substrate (30% liquid fractions DCM and 70% enzyme-treated SFDCM) when compared with the control digester treating a similar mixed substrate with inactivated enzyme addition. The results indicate that direct physical contact of enzyme molecules and organic material in DCM prior to AD, without the intervention of extracellular enzymes from the indigenous microorganism population, was needed in order to increase methane yields.

Keywords: biogas; mixed enzymes; pre-treatment; incubation; manure

1. Introduction

Manure has great potential as a substrate for anaerobic digestion (AD) in Europe.[1] AD is a multistep process that consists of hydrolysis, acidogenesis, acetogenesis and methanogenesis stages.[2] Hydrolysis, the first step of AD, is known as a limiting step when solid waste or animal slurry with high fibre concentration is treated.[3,4] Moreover, the degradability of manure under anaerobic conditions is only around 40–50% of the total solids (TS) due to its high fibre content.[5] There are several pre-treatment methods to increase the biodegradation rate of organic material, including physical pre-treatment, physico-chemical pre-treatment, chemical pre-treatment and biological pre-treatment.[6] Biological pre-treatment, such as enzyme application, has many advantages, such as a low energy input, no chemical requirement, and it is also environmentally friendly.[6]

Enzyme application promotes the hydrolysis of complex organic polymers to molecules of lower molecular weight, thus making them available for utilization by microorganisms.[7] Studies to investigate the addition of enzymes to improve the biodegradation rate of various organic materials have been carried out previously. Pre-treatment application of a commercially available trizyme (cellulase, α-amylase and protease) on wheat grain at 37 °C for 24 h prior to AD increased methane production by 7–14%.[8] Enzyme addition has been shown to have a significant effect on the solubilization of wheat grass but had no additional positive effect on methane yield and volatile solids (VS) reduction when the treated material was subjected to AD.[9] Enzyme addition after steam pre-treatment with a catalyst (H_3PO_4 and NaOH) increased the methane yield of digested fibre fractions by 2.0 ± 0.5 and 1.7 ± 0.4 m^3 CH_4 (t w/w), respectively, compared with catalysed steam pre-treatment alone.[10] However, to our knowledge, there is still lack of information about application of mixture of enzymes (ME) to continuous feed digester with dairy cattle manure (DCM) as a substrate.

Increasing methane production per unit digester volume can be achieved by increasing the dry matter of the substrate through the addition of solid manure fractions.[11] Enzyme pre-treatment in the solid fractions of dairy cattle manure (SFDCM) is attractive, since the recalcitrant fibre of manure is mainly in the solid fractions. The effect of enzyme application is dependent on many factors, including the substrate, incubation period, system

*Corresponding author. Emails: soeta@undip.ac.id; sutaryoundip@yahoo.com

configuration and environmental conditions (e.g. temperature and pH);[6] therefore, this work examines direct enzyme application experiments in DCM and the SFDCM, under mesophilic and thermophilic conditions and with enzymatic pre-treatment of substrate prior to AD. In addition, because Yang et al. [12] have found that a ME application had a better effect on sludge solubilization than a single-enzyme application, it was decided that it would be of interest to apply ME to DCM and SFDCM rather than use a single enzyme. This study was motivated by the abundance of manure in the EU [1] and the possibility of increasing the methane yield of animal manure through AD by ME application.

2. Materials and methods

2.1. Experimental design

Three experiments were conducted to investigate the effect of ME application on the methane yield of DCM during AD. The first experiment was a ME application to substrate at thermophilic (50 °C) digesters. There were two kinds of ME application in the first experiment: ME addition to DCM with immediate feeding to digesters and addition of ME to DCM in an enzymatic pre-treatment reactor prior to AD. The second experiment consisted of addition of ME to DCM and immediate feeding to mesophilic (35 °C) digesters. The third experiment was the addition of ME to the SFDCM followed by an incubation period prior to mixing with liquid fractions of DCM and feeding to thermophilic digesters. The detailed experimental set-up can be seen in Table 1.

In the control experiments, the inactive ME were deactivated by autoclaving at 121 °C for 30 min according to Yunkin et al.[13] Enzymes were obtained from a commercial enzyme manufacturer (Novozymes A/S, Bagsvaerd, Denmark). The enzyme specifications are shown in Table 2.

Dairy cows are normally fed mainly with roughage which has a high concentration of lignin complexed with cellulose in the organic matter,[14] thus the enzymes in this study were selected based on their ability to hydrolyse cellulose and to break down plant cell walls, and also on their temperature and pH profiles. Experiments were run for 42 days (three times hydraulic retention time (HRT) for the first and third experiments and 2.1 times HRT for the second experiment). During the experiments, all digesters were fed once a day by first removing an amount of digested material equal to the amount of feed material from a port at the base of each digester. The digesters were then fed through a tube, the outlet of which was submerged under the substrate level to avoid air ingress during the feeding process.

2.1.1. ME application to thermophilic digesters

Three identical continuously fed digesters (R1, R2 and R3) with 10 L total capacity and 7 L working capacity were used in this experiment. R_{pt} (enzymatic pre-treatment reactor)

Table 1. Experimental design.

Exp. no.	Digester	Substrate	HRT (days)	Digester temperature (°C)	Enzyme application (g ME/g VS)	Enzyme	Organic loading rate (kg VS/m^3/day)
1	R_{pt}[a]	DCM	3	50	0.025	Active	30.87
	R1	Effluent from R_{pt}	14	50	–	–	6.40
	R2	DCM	14	50	0.025	Active	6.61
	R3	DCM	14	50	0.025	Inactive	6.61
2	R4	DCM	20	35	0.03	Active	3.25
	R5	DCM	20	35	0.03	Inactive	3.25
3	R6	70% SFDCM and 30% liquid fraction of DCM	14	50	0.025 (SFDCM only treated and 35 °C pre-incubation for 20 h)	Active	6.94
	R7	70% SFDCM and 30% liquid fraction of DCM	14	50	0.025 (SFDCM only treated and 35 °C pre-incubation for 20 h)	Inactive	6.94

[a]Enzymatic pre-treatment reactor.

Table 2. Enzyme specifications.

Name	Activity	Units	Temperature range (°C)	pH range
NS 81215	Pectate lyase	3000 APSU/g (alc. pectinase standard units)	25–65	6–9
NS 81216	Cellulase	16,000 HCU/g (high cellulose units)	35–65	3.5–7.5
NS 81218	Cellulase	90 EGU/g (endoglucanase units)	40–65	5–9
NS 81220	Protease	2.5 AU-A/g (protease unit AU-A)	25–65	7–11

had a working capacity of 2.1 L. Inoculum was sourced from the active commercial biogas digester at Research Centre Foulum, Denmark, which operates at a thermophilic temperature (52 °C). pH, TS and VS were 7.78%, 4.4% and 3.3%, respectively. The commercial digester treats pig manure, cattle manure, maize silage and industrial by-products. The substrate was DCM from the lactation period and was collected from a slurry storage pit at Research Centre Foulum, Denmark. Manure was collected every two weeks and was kept at room temperature in a sealed 50 L plastic barrel. The experiment was started by filling R1, R2 and R3 with 6.5 L of inoculum and 0.5 L of DCM on the first day. Inoculum was not used for R_{pt}; instead DCM and active ME were added from the first day onwards.

During normal operation, R_{pt} was fed with DCM and active ME. Effluent from this digester was used to feed R1. R2 was fed with DCM and active ME, while R3 was fed with DCM and inactive ME and served as a control. The enzyme mixture consisted of equal proportions (w/w) of enzyme NS 81215, enzyme NS 81216 and enzyme NS 81218. The purpose of the addition of ME to the enzymatic pre-treatment reactor in this study is to examine the possibility of ME pre-treatment of DCM prior to digestion and to investigate how that can increase methane yield.

2.1.2. *ME application to mesophilic digesters*

This study was conducted in two identical continuously fed digesters with 10 L total capacity and 7 L working capacity. Inoculum was from the post-digestion tank at Research Centre Foulum, which operates at 29.7 °C, with pH, TS and VS of 7.4%, 3.8% and 2.6%, respectively. Substrate was collected every two weeks from the same storage pit as described in the first experiment. To start the experiment, the digesters were filled with 6.65 L of inoculum and 0.35 L of DCM, and normal feeding continued from then onwards.

Due to the lack of a positive effect of a 0.025 g/g VS substrate ME concentration in the first experiment, the ME concentration was increased to 0.03 g/g VS substrate and enzyme NS 81220 (protease) was included. The treatment in R4 was ME addition of enzyme NS 81215, enzyme NS 81216, enzyme NS 81218 and enzyme NS 81220 in equal proportions (w/w). Enzyme NS 81220 was mixed with DCM separately after the other enzymes had been added to prevent proteolytic degradation of enzymes NS 81215, NS 81216 and NS 81218. Treatment R5 served as a control and was fed with DCM and inactive ME.

2.1.3. *ME application to SFDCM*

The third experiment was conducted in two identical intermittently stirred tank digesters, with 6.2 L total capacity and a working capacity of 4.2 L. The mixing system operated at 60 revolutions per minute (rpm) for 15 min for every hour.

Inoculum was also taken from the active commercial biogas digester at Research Centre Foulum, as described for Experiment 1, but the pH, TS and VS were 7.7%, 4.6% and 3.7%, respectively.

Substrate was collected on one single occasion. Normal feeding was performed during the start-up period of three weeks using DCM as a substrate. SFDCM was obtained by manual separation of manure using a sieve (500 μm, serial number 5564470 D-42759, Haan, Germany). SFDCM and liquid fractions of DCM were kept at −20 °C until used. The substrate properties can be seen in Table 3. The substrate added to the digesters was a mixture of the SFDCM and liquid fractions of DCM (70:30 w/w, respectively). Equal proportions (w/w) of enzyme NS 81215, enzyme NS 81216 and enzyme NS 81218 were diluted with deionized water 1:5 (w/w) prior to mixing with the SFDCM. A kitchen mixer (National Panalux, PHM-25) was used at a low speed to mix the SFDCM and ME for 2 min. Pre-incubation of the SFDCM with ME was performed at 35 °C for 20 h. During this process, the SFDCM mixed with ME was placed in 0.5 L sealed plastic bottles and turned continuously at 60 rpm using a rotating mixer (model SC-2290D Shin Myung Servo Co Ltd, Korea). Following pre-incubation, the SFDCM was mixed with the liquid fraction of DCM prior to use as substrate to the digesters. R6 served as a treatment which was fed with substrate and active ME, and R7 was the control which was fed with substrate and inactive ME.

2.2. *Analytical methods*

Biogas was collected daily using aluminium-coated gas bags and measured using a syringe (model S-500, Hamilton Co, Reno, Nevada, USA) for Experiment 1, as described by,[15] and for the second and third experiments, it was measured using an acidified water displacement method. Gas samples were analysed for CO_2 and CH_4 content using a Perkin Elmer Clarus 500 gas chromatograph equipped with a thermal conductivity detector and a Turbomatrix 16 Headspace auto sampler. Methane and CO_2 were isolated using a $12' \times 1/8''$ Hayesep Q 80/100 column, helium (He) was used as the carrier gas at 30 mL/min, and the injection port, oven and detector temperatures were 110 °C, 40 °C and 150 °C, respectively.

Volatile fatty acids (VFA) (C_2–C_5) were determined by means of a gas chromatograph (Hewlett Packard 6850A) with a flame ionization detector (FID). The column was an HP-INNOWax, 30 m × 0.25 mm × 0.25 μm. The carrier gas was He. The temperature of the column was gradually increased from 110 to 220 °C at the rate of 10 °C per minute.

TS was determined by drying at 105 °C for 24 h. Ash was determined by combusting the dried samples at 550 °C for 5 h, and VS was calculated by subtracting the ash weight from the TS. Total nitrogen was analysed using the Kjeldahl standard method [16] and a Kjell-Foss 16,200 auto analyser (Foss Electric, Hillerod, Denmark). Total

Table 3. Substrate properties.

Exp.no.	Substrate	pH	VFA					TAN	TS (%)	VS (%)
			Acetic acid (mg/L)	Propionic acid (mg/L)	Isobutyric acid (mg/L)	Butyric acid (mg/L)	Isovaleric acid (mg/L)	Valeric acid (mg/L)			
1	DCM	7.2 ± 0.2	7777 ± 1868	2491 ± 733	310 ± 48	1455 ± 315	477 ± 60	165 ± 66	n.a	11.4	9.3
2	DCM	6.9 ± 0.1	4197 ± 1197	1326 ± 428	133 ± 47	491 ± 183	205 ± 62	87 ± 36	2110	7.8	6.5
3	SFDCM[a]	7.56	57 ± 21	23 ± 13	Ud	Ud	Ud	3 ± 5	1540	14.4	12.9
	SFDCM[b]	7.5 ± 0.6	3010 ± 735	923 ± 192	155 ± 36	348 ± 162	227 ± 61	71 ± 35	1490	14.3	12.7
	SFDCM[c]	7.5 ± 0.5	2512 ± 690	777 ± 168	139 ± 32	291 ± 138	207 ± 49	57 ± 29	1460	14.3	12.6

Note: n.a, not available and ud, undetected.
[a]Prior to pre-treatment.
[b]Pre-treatment with active ME.
[c]Pre-treatment with inactive ME.

ammonia nitrogen (TAN) was measured colorimetrically at 690 nm with a Merck spectrophotometer (NOVA 60, NH_4^+ test 1.00683.0001). pH was measured using a pH meter (Metrohm AG, CH-9101, Herisau, Switzerland). Gas composition, VFA, TAN and pH value were analysed twice a week. TS and VS were analysed once a week. Data were analysed using ANOVA and *T*-test with 95% confidence level. R® software was employed. Prior to analysis, data were checked for normality and homogeneity. In the case of non-normally distributed data in some variables, transformation was applied. Data from R_{pt} were not included in the statistical analysis due to their incomparability with the other treatments.

3. Results and discussion

3.1. ME application to thermophilic digesters

There was no significant effect ($p > 0.05$) on the methane yield of DCM following ME addition to the thermophilic digester (Table 4). Mean methane yields were 136 ± 15 L/kg VS in R2 and 135 ± 13 L/kg VS in R3 (control). The methane yields throughout the experiment are shown in Figure 1(a). The lack of a positive effect on methane yield may be due to the degradation of the ME by microbial activity in the digester, since it was a direct addition of ME to DCM in AD;[17] hence the enzymes were not able to promote hydrolysis of the DCM optimally. However, a significant effect on the methane yield ($p < 0.05$) was found in R1 which was fed DCM that had been incubated for three days with ME addition at 50 °C (Table 4). The methane yield from digester R1 was 141 ± 14 L/kg VS. R1 was operating at the same HRT as the control digester (R3) and methane production in R1 was increased significantly ($p < 0.05$), about 4.44% higher than that of R3. Methane yield from R_{pt} was 5 ± 1 L/kg VS, thus total methane yield from R1 and R_{pt} (17 days HRT in total) was 146 L/kg VS, an increase of 8.33% compared with control. A positive effect on the methane yield of R1 compared with R3 may be caused by the synergistic effect of ME addition and solubilization and degradation of particulate matter in R_{pt}. Therefore, further experimentation is needed to confirm a positive effect of ME addition using similar process conditions. This work did not aim to evaluate the effect of ME addition to the individual organic components in DCM, although two-thirds of ME in Experiments 1 and 3 had cellulase activity and one-third had pectase lyase activity. Raju et al. [18] found that cellulose in manure is about 21% of TS in DCM and 31% of TS in pig manure, while in grasses, the feed component of dairy cattle, 2–10% of TS in the cell wall is pectin.[19] Application of ME in the enzymatic pre-treatment digester also gave a positive effect on the biogas composition, thus methane concentrations in R1 and R3 were 73 ± 2% and 64 ± 1%, respectively. The combination of R_{pt} and R1 can be considered as a two-stage process, although direct comparison of the combined yield with that of R3 is not possible

Table 4. Process parameters.

		Experiment 1				Experiment 2			Experiment 3	
Parameters	Units	R_{pt}	R1	R2	R3	R4	R5	R6	R7
Methane yield	L/kg VS	$5 \pm 1^{*}$	141 ± 14^{a}	136 ± 15^{b}	135 ± 13^{b}	174 ± 10^{c}	172 ± 10^{c}	130 ± 12^{d}	125 ± 11^{e}
TAN	mg/L	n.a	n.a	n.a	n.a	2380^{a}	2430^{a}	1660^{b}	1710 ± 0.3^{b}
Total VFA	mg/L	$19,809 \pm 1111^{*}$	4308 ± 1144^{a}	4465 ± 1098^{a}	4443 ± 1210^{a}	133 ± 44^{b}	147 ± 32^{b}	90 ± 12^{c}	102 ± 33^{c}
VS reduction	%	–	25.6 ± 7.7^{a}	26.2 ± 5.5^{a}	25.3 ± 5.2^{a}	31.9 ± 5.0^{b}	29.4 ± 6.5^{b}	32.7 ± 5.9^{c}	31.4 ± 7.2^{c}
pH		$7.4 \pm 0.3^{*}$	8.3 ± 0.2^{a}	8.3 ± 0.2^{a}	8.3 ± 0.2^{a}	7.7 ± 0.04^{b}	7.7 ± 0.06^{b}	7.9 ± 0.06^{c}	7.9 ± 0.05^{c}

Note: Values in each row within each experiment followed by the same superscript letter are not significantly different ($p > 0.05$). n.a, not available.
*Data were not compared.

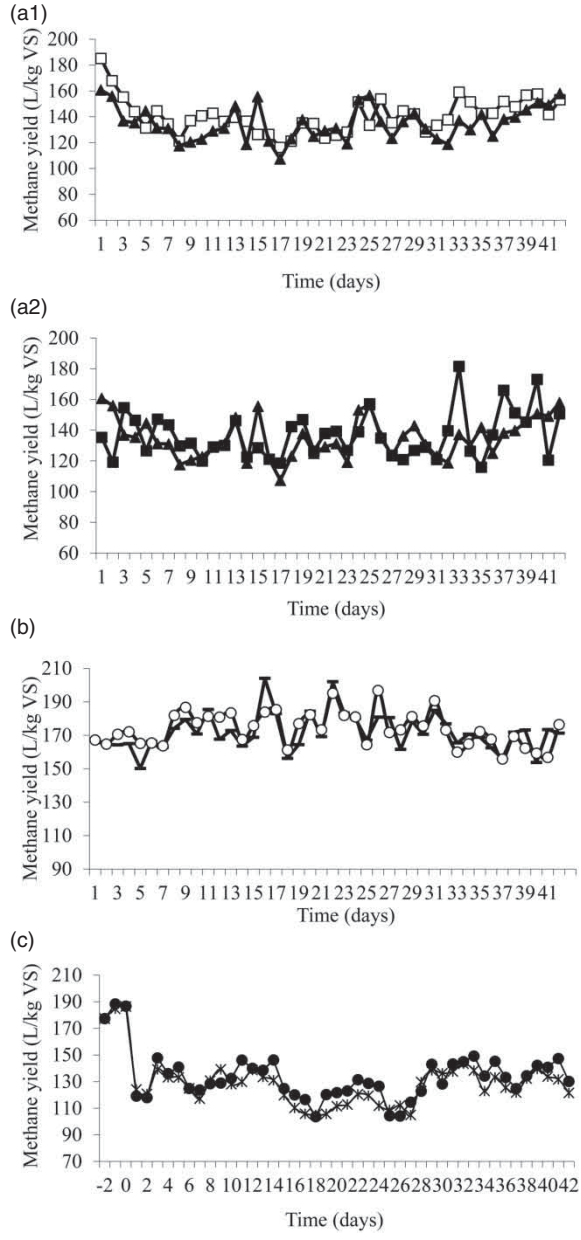

Figure 1. Methane yield: (a) Experiment 1, (b) Experiment 2 and (c) Experiment 3 (□: R1, ■: R2, ▲: R3, ◯: R4, –: R5, ●: R6 and ∗: R7).

due to the difference in retention time. However, a higher methane concentration in R1 is in accordance with,[7] who found a methane concentration of 73.4% in the second stage of a two-stage mesophilic (37 °C) digester using enzymatically treated spent grain as substrate, and 66.7% in the control (using untreated spent grain as substrate). The increased methane concentration in R1 could be because the acetogenic and methanogenic microorganisms are more dominant in R1 than other microorganisms, and therefore methane concentration from this digester was higher than that from R3. Gunaseelan [20] reported that in two-stage digesters, the hydrolysis and acidification processes are

accomplished in the first stage, while acetogenesis and methanogenesis take place in the second-stage digester. Total VFA concentration in R_{pt} was higher than that in R3, while pH was lower than the values measured in R3. AD is a multi-stage process including hydrolysis, acidogenesis, acetogenesis and methanogenesis steps. Volatile fatty acids, ammonia, CO_2, H_2S and other by-products are produced during the acidogenesis step by acidogenic (or fermentative) microorganisms.[13] The high VFA and CO_2 concentration and low pH value in R_{pt} were, therefore, indicative of high hydrolytic and acidogenic activity.

No significant effect ($p > 0.05$) on methane concentration was found in R2 and R3. Methane concentration was $64 \pm 2\%$ in R2 and $64 \pm 1\%$ in R3. Furthermore, the addition of ME to DCM in the thermophilic digester gave no significant effect ($p > 0.05$) in VS reduction, total VFA and pH. The values of these parameters are shown in Table 4. No positive effect on VS reduction in R1 compared with R3 can be attributed by the difficulty to get a representative sample from the laboratory scale digester and the dissimilarity in methane production in both digester was not considered large enough for the effect on VS reduction to be significant. This assumption is supported by the high standard deviation (5–8%) of VS reduction data (Table 4).

Total VFA concentration in the digestate from the first experiment was higher (Table 4) than that of the other experiments. The dissimilarity may be due to the higher total VFA the substrate in this experiment (Table 3) than in other experiments. This experiment was carried out during the winter season, while other experiments were carried out in spring and summer. Therefore, it is possible that the bedding material in the manure was higher in this experiment than in other experiments. This fact was strengthened by a higher VS content in the substrate for the first experiment compared with the other experiments. Values of VS in the substrate were 9.3 ± 0.3, 6.5 ± 0.5 and $8.8 \pm 1.7\%$ in the first, second and third experiments, respectively.

3.2. ME application to mesophilic digesters

The mean methane yields of mesophilic digesters subjected to addition of ME were 174 ± 10 L/kg VS in the treatment (R4) and 172 ± 10 L/kg VS in the control (R5). The yields during the course of the experiment are shown in Figure 1(b). The result was not significant ($p > 0.05$). In this study, methane yield and VS reduction were higher (Table 4) at mesophilic than at thermophilic temperatures (Experiment 1). This can be explained by a longer HRT and a higher ME concentration in Experiment 2 compared with the other experiments, and different weather conditions and biogas collection methods may also have had a role to play. Sarapatka [21] reported that season had an effect on biogas production per large animal unit per day, whereby the biogas production was higher in the summer period than in a transitional and a winter period. This result is also higher than the ultimate methane yield from DCM of 148 ± 41 L/kg VS reported by Møller et al.[14] In addition, the ultimate methane yield from various animal slurries can vary. This dissimilarity may be due to differences in manure composition, as reflected in the differences in the organic matter composition of manure, including VFA, proteins, lipids, carbohydrates and lignin.[22] The lack of positive effect of direct ME addition in this study is in accordance with that of Romano et al.,[9] where direct enzymes were applied in AD using wheat grass as the substrate. The results shown here indicate that extracellular hydrolytic enzymes from the microorganisms already present in the digesters were sufficient for the enzymatic hydrolysis of the available material and therefore were not the limiting factor.[9]

3.3. ME application to the solid fraction of DCM

The methane yields of Experiment 3 are shown in Figure 1(c). Methane yield decreased immediately following treatment due to a different loading rate: during the start-up period the digesters were fed with DCM, while during treatment the substrate was 70% SFDCM and 30% liquid fraction of DCM (Table 1). The decreased yield can be attributed to a potentially lower yield of the solid fraction, due to the proportion of the VS which is easily degradable being lower in the solid fraction than in the whole manure.[23] The solid fractions contribute a considerable proportion of the VS of the solid–liquid mixed manure. VS values of the substrates were 6.1% and 9.7% in the start-up and treatment periods, respectively, while the VS of the solid fractions and liquid fractions of DCM were 12.9% and 3.0%, respectively. Another factor might be a lower nutrient concentration in the substrate during treatment. Møller et al. [11] reported that, based on VS composition, the protein and lipid concentration in the solid manure were slightly lower than in whole manure. Due to the protein and lipid concentrations in the substrate in the treatment period being lower than in the start-up period, methane yield per VS of the substrate during the treatment period was lower than that in the start-up period. This explanation is strengthened by the fact that the total nitrogen (% of VS) of substrate in the third experiment was 4.6% and 3% during the start-up and treatment periods, respectively.

This study found a significant effect ($p \leq 0.05$) on the methane yield of the treatment digester (R6) (130 ± 12 L/kg VS) when compared with the methane yield of the control digester (R7) (125 ± 11 L/kg VS), corresponding to 4.15% increased methane yield. In this work, the abundance of acetic acid in the treated substrate (Table 3) could explain the increased methane yield, since acetic acid can be utilized by methanogenic bacteria to form methane directly.[10] An increased acetogenesis would require an increase in the precursors of acetic acid (e.g. propionate, butyrate, monosaccharides, amino acids, etc.), which would in turn indicate an increased hydrolysis as a result of ME addition. Moreover, a similar result was also found in the concentration of propionic and butyric acid concentrations.

When compared with the control, the treated solid fraction of DCM had 19% higher propionic acid concentration and 20% higher butyric acid concentration (Table 3). Even though the aim of this study was not to evaluate the individual target compounds of ME addition (see Section 3.1), total VFA of treated SFDCM with active ME addition was increased by about 20% compared with SFDCM treated with inactive ME addition. This fact could be seen as evidence of hydrolytic activity on the cellulose component in DCM by the cellulase enzymes in ME, since cellulases were the greater part of the enzyme mixture and cellulose in DCM has been shown to be approximately 21% of DCM TS.[18] However, the high cost of ME application in AD compared to the extra methane yield following ME application to the SFDCM of around 4.15% found in this study may still be a limiting factor for commercial applications, even though some studies regarding genetic engineering to produce low-cost enzymes are addressing this issue.[24]

The positive effect of ME addition on methane yield in the pre-incubation of the solid fraction of DCM indicates that a direct physical contact between the enzyme molecules and the organic material in the DCM prior to AD is required, without the intervention of extracellular enzymes from microorganisms in the digester.[6]

4. Conclusions

ME addition to DCM followed by pre-incubation at 50 °C for three days and also to SFDCM prior to a pre-incubation step at 35 °C for 20 h significantly increased the respective methane yields. Methane yield after pre-incubation of DCM was 4.44% higher than in the control digester with inactive ME addition, while methane yield from the digester fed with 30% liquid fractions DCM and 70% enzyme-treated SFDCM was 4.15% higher than the control. No significant effect was seen when ME were added to DCM and then fed directly to either mesophilic or thermophilic AD processes. In order to increase the methane yield of DCM by the addition of enzymes, the enzymes need to be added in an enzymatic pre-treatment step.

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